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TECHNICAL MANUSCRIPT 476

**SPORICIDAL EFFECT
OF PERACETIC ACID VAPOR**

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SEPTEMBER 1968

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**DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701**

TECHNICAL MANUSCRIPT 476

SPORICIDAL EFFECT OF PERACETIC ACID VAPOR

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COMMODITY DEVELOPMENT AND ENGINEERING LABORATORY**

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ABSTRACT

The activity of peracetic acid (PAA) vapor at 20, 40, 60, and 80% relative humidity (RH) and 25 C was determined against Bacillus subtilis var. niger spores on paper and glass surfaces. Appreciable kill occurred within 10 minutes when the spores were exposed to 1 mg PAA per liter and the RH was 40% or higher. The rate of kill decreased with RH from the optimum at 80% RH to only a slight effect at 20% RH. Spores on an impermeable surface appear to be more difficult to kill than those on a porous one, probably because the cells tend to pile up on an impermeable surface and the vapor penetrates poorly through the layer of covering cells.

I. INTRODUCTION

Numerous studies¹⁻⁶ have shown that peracetic acid (PAA) in aqueous solution is an effective germicide against a wide spectrum of microorganisms. On the other hand, information about the vapor-phase bactericidal activity of PAA is very limited and seems to be confined to the very brief study by Greenspan, Johnsen, and Trexler.⁷

The corrosiveness of PAA has limited its practical application as a disinfectant. However, because PAA itself is not adsorbed onto surfaces and its decomposition products (acetic acid, water, and oxygen) are non-toxic and free-rinsing, PAA has found wide application as a surface decontaminant for foods.^{8,7,8} Spraying dilute solutions of PAA until a fog forms has been an effective means of sterilizing equipment used in gnotobiotic studies.^{9,10}

The accumulation of liquid PAA and moisture produced from spraying a dense fog may be undesirable when one does not want to wet surfaces because of corrosion or other damaging effects. We therefore investigated the possible use of PAA vapor. Because of the extreme reactivity and considerable volatility of PAA, the study reported here was undertaken to determine the effectiveness of PAA vapor against bacterial spores, both on porous and impermeable surfaces, at various relative humidities (RH).

II. MATERIALS AND METHODS

A. PREPARATION OF TEST SAMPLES

An aqueous stock spore suspension of Pacillus subtilis var. niger was used to contaminate filter paper discs (5/8-inch diameter) and glass squares (0.5 by 0.5 inch). The contaminated samples were conditioned for 4 days in desiccators maintained at 25 C and at constant humidities of 80, 60, and 40% by saturated salt solutions of ammonium sulfate, sodium bromide, and chromic acid, respectively. Samples were conditioned at 20% RH in the laboratory during the winter months when the ambient RH was not only low but also constant as indicated by a hygrothermograph.

B. EXPOSURE TO PAA

The 86-liter test chamber (Fig. 1) was fabricated for vapor-phase decontaminant studies. Prior to spraying PAA, the RH within the test chamber was adjusted to the same RH used to precondition the samples, by

either spraying water into the chamber to raise the RH or blowing dry air in to lower it. No adjustment was needed for tests conducted at 20% RH because the ambient RH of the laboratory was the same as that desired in the test chamber. The RH within the test chamber was monitored with a humidity-sensing element.*

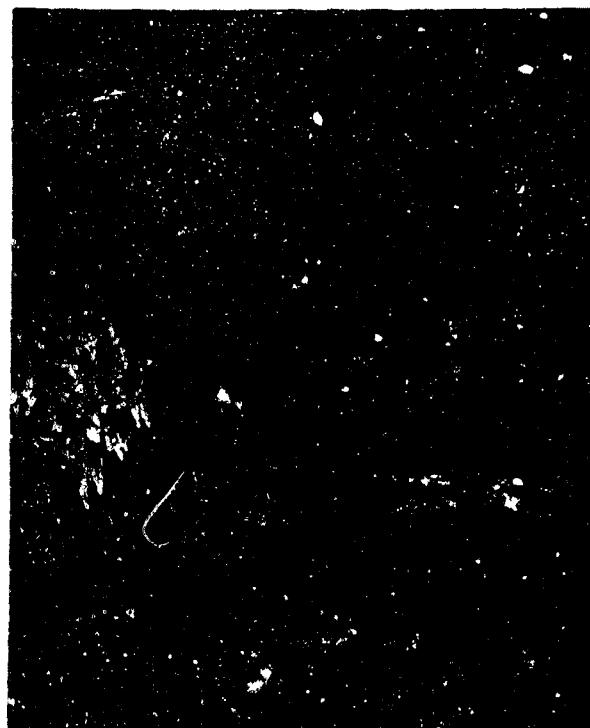


Figure 1. Test Chamber.

* El-tronics, Inc., Warren, Pa.

The commercial grade of PAA solution is composed of approximately 40% peracetic acid, 5% hydrogen peroxide, 39% acetic acid, 1% sulfuric acid, and 1.5% water, by weight. A direct spray UCTL atomizer¹¹ was used to spray 0.5 ml of the undiluted solution into the test chamber. To allow time for the vapor concentration to stabilize, the test samples were not exposed to the vapor until 30 minutes after spraying (note rapid initial drop in concentration in Figure 2). Furthermore, the fan in the test chamber was run throughout the test period to insure uniform distribution of the PAA vapor. For each exposure period, 16 samples (eight contaminated paper, mounted on pins; eight glass, laid on wire screen) were placed on an aluminum tray and quickly shoved into the middle of the chamber (Fig. 1). All samples were exposed to PAA vapor (about 1 mg per liter) at 25±2 C and the same RH as that used for conditioning the samples. Individual exposure periods ranged from 1.25 to 80 minutes.

C. PAA CONCENTRATION

The concentration of PAA in the chamber was determined at periodic intervals during each exposure period by drawing 0.5 liter of the chamber air through 20 ml of 20% buffered potassium iodide. The optical density was measured at 500 m μ with a spectrophotometer and the concentration was read from a standard curve prepared by plotting optical densities against known weights of PAA. This method of determining concentrations of PAA is specific.

D. METHOD OF ASSAY

After exposure to PAA vapor, the samples were transferred to sterile stoppered plastic tubes containing 10 ml of 0.01% sodium thiosulfate solution (to neutralize excess PAA) and about 0.5 g of sand (to aid in removal of spores) and then shaken vigorously. To insure that an estimate of the number of organisms could be obtained regardless of population size for each exposure period, duplicate 2.5- and 1-ml portions from each tube were plated directly and the remainder was used for serial dilutions. Pour plates were prepared with trypticase soy agar. Plate counts were made after 72 hours' incubation at 32 C. Conditioned samples not exposed to PAA vapor were also assayed at each exposure period.

The accuracy of counting a small population is increased by plating a large portion of the sample. Confidence that sterility is being attained is also probably increased when no colonies appear on any of the plates prepared from over 70% of the suspending fluid of the sample.

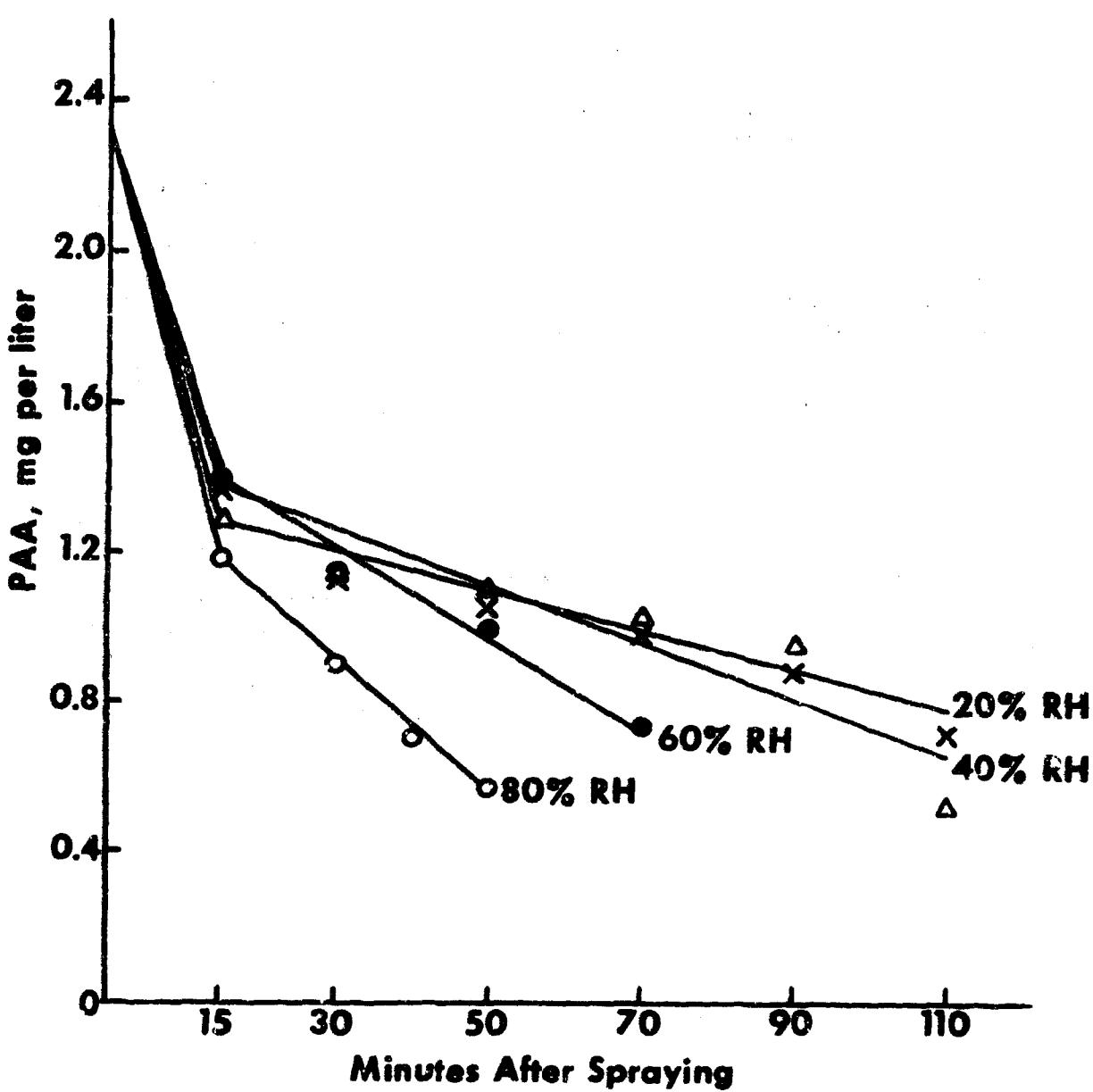


Figure 2. Concentration of PAA Vapor in Test Chamber as a Function of Time.

III. RESULTS AND DISCUSSION

The results are summarized in Table 1. Because an exceptionally high value occasionally appeared among the individual sample counts for given conditions, otherwise of the same order of magnitude, the data are expressed by geometric rather than arithmetic means to more truly represent the relationships among the samples. For many of the 16 test samples assayed per exposure period, no organisms were recovered from the 70% of the sample plated. The frequency of this occurrence is also given in Table 1 as another indication of vapor activity.

The results show that most of the spore population was killed with PAA vapor within a few minutes when the RH was 40% or higher. At 20% RH, however, there was only a slight spore reduction on paper and none on glass after 80 minutes' exposure to the vapor.

The apparently faster kill of spores on paper than on glass is probably due to spore distribution. The spores in a drop of water spread over a large surface area when placed on a porous material and thus are not likely to pile upon one another as the liquid evaporates. When a similar drop of suspension begins to dry on an impermeable surface, however, there is a tendency for the organisms to collect and pile up at the periphery of the liquid. Such a pileup of cells will provide protection for the spores underneath from the lethal action of a poorly penetrating vapor like PAA.

The determination of D values (decimal reduction time) for data obtained in the latter situation would not be meaningful because the death curve is nonlinear; there is a rapid initial death rate, then a decrease and leveling off of the curve. The concentration of PAA (Fig. 2) gradually decreases with time, but the decrease is not sufficient to account for the slower rate of kill with time. Greenspan et al.⁸ concluded from their studies that PAA vapor-phase sterilization was only dependable with clean surfaces. Trexler and Reynolds¹⁰ attributed dirt as the cause for their difficulty in sterilizing animal cages with a fog of PAA. The surfaces used in the present study were clean, but the high concentration of spores on the small sample area of the impermeable surface may have had an effect similar to that of dirt. The outer layer of spores killed during the initial exposure may physically protect the remaining viable spores. Because PAA vapor apparently does not penetrate well, its power as a germicide seems to be limited to exposed microorganisms.

The results of this study indicate that, at RH between 40 and 80%, PAA vapor can appreciably reduce spore contamination on both porous and impermeable surfaces within 10 minutes. However, the optimum sporicidal activity occurs at 30% RH, and no appreciable activity occurs at a low RH (20%). The level of microbial contamination and the cleanliness of the surface as well as RH are undoubtedly factors that determine whether sterility is achieved by treatment with PAA vapor.

TABLE I. EFFECTIVENESS OF PERACETIC ACID VAPOR^a/ AGAINST *B. SUBTILIS* VAR. *NIGER* SPORES
ON PAPER AND GLASS AT VARIOUS RELATIVE HUMIDITIES

Exposure to PAA, min.	Number Samples/ sterile ^c / (of 16)	80% RH		60% RH		40% RH		20% RH	
		Number Samples/ sterile ^c / (of 16)		Org. per Sample ^b		Number Samples/ sterile ^c / (of 16)		Org. per Sample ^b	
		Surfice	Sample	Org. per Sample ^b	Sample	Surfice	Sample	Org. per Sample ^b	Sample
Paper	d/	816,000	831,000	-	-	658,000	-	275,000	-
	1.25	676	0	-e/	-	-	-	-	-
	2.5	1	5	1,390	0	-	-	-	-
	5	<1	12	14	2	7,270	0	-	-
	10	<1	14	2	7	24	2	171,000	0
	20	0	16	1	8	7	1	117,000	0
	40	-	-	<1	12	<1	8	129,000	0
	80	-	-	-	-	<1	13	26,000	0
Glass	d/	813,000	971,000	-	-	709,000	-	290,000	-
	1.25	5	7	-	-	-	-	-	-
	2.5	2	7	216	1	-	-	-	-
	5	<1	13	88	2	33,600	0	-	-
	10	<1	13	38	0	1,530	0	218,000	0
	20	<1	10	4	6	2,330	0	153,000	0
	40	-	-	2	7	623	0	327,000	0
	80	-	-	-	-	143	-	267,000	-

- a. Approximately 1 mg PAA per liter.
- b. Each entry is a geometric mean based on 16 samples except at 20% RH, based on 8.
- c. Based on plating 70% of each sample.
- d. Control, no exposure to PAA.
- e. - = not tested.

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